Free Radical Research, Volume 38 Number 6 (June 2004), pp. 541–551

Taylor & Francis
healthsciences

Dual Role of Oxidized LDL on the NF-KappaB Signaling Pathway

FANNY ROBBESYN, ROBERT SALVAYRE and ANNE NEGRE-SALVAYRE*

INSERM U-466, CHU Rangueil, 31403 Toulouse, Cedex 4, France

Accepted by Professor Helmut Sies

(Received 10 September 2003; In revised form 15 December 2003)

Atherosclerosis is a slowly evolutive age-linked disease of large arteries, characterized by a local lipid deposition associated with a chronic inflammatory response, leading potentially to acute plaque rupture, thrombosis and ischemic heart disease. Atherogenesis is a complex sequence of events associating first expression of adhesion molecules, recruitment of mononuclear cells to the endothelium, local activation of leukocytes and inflammation, lipid accumulation and foam cell formation. Low density lipoproteins (LDLs) become atherogenic after undergoing oxidation by vascular cells, that transform them into highly bioreactive oxidized LDL (oxidized LDLs). Oxidized LDLs are involved in foam cell formation, and trigger proatherogenic events such as overexpression of adhesion molecules, chemoattractant agents growth factors and cytokines involved in the inflammatory process, cell proliferation and apoptosis. Moreover, this toxic effect of oxidized LDLs plays probably a role in plaque erosion/rupture and subsequent atherothrombosis.

Several biological effects of oxidized LDLs are mediated through changes in the activity of transcription factors and subsequently in gene expression. Oxidized LDLs exert a biphasic effect on the redox-sensitive transcription factor NF-κB, which can be activated thereby up-regulating proinflammatory gene expression, such as adhesion molecules, tissue factor, scavenger receptor LOX-1. On the other hand, higher concentrations of oxidized LDLs may inhibit NF-κB activation triggered by inflammatory agents such as LPS, and may thereby exert an immunosuppressive effect. This review is an attempt to clarify the mechanism by which oxidized LDLs may up- or down-regulate NF-κB, the role of NF-κB activation (or inhibition), and the consequences of the oxidized LDLs-mediated NF-κB dysregulation and their potential involvement in atherosclerosis.

Keywords: Oxidized LDL; NF-κB; Inflammation; Atherosclerosis; Reactive oxygen species

Abbreviations: Oxidized LDLs, oxidized low density lipoproteins; ROS, reactive oxygen species; NF-κB, nuclear factor kappaB; IκB, IkappaB; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intracellular cell adhesion molecule-1; PPARs, peroxisome-proliferator-activated receptors

INTRODUCTION

Atherosclerosis with its related complications, heart attack, stroke and peripheral vascular diseases, is a prevalent cause of morbidity and mortality in western countries. Atherogenesis is characterized by cholesteryl esters accumulation in atherosclerotic plaques, associated with several cellular effects, such as endothelial activation, migration of mononuclear cells, local chronic inflammatory process, smooth muscle cell proliferation and cell death. This may lead to acute complications, such as plaque erosion or rupture and thrombotic events.[1–3]

Low density lipoproteins (LDLs) are involved in the pathogenesis of atherosclerosis after undergoing oxidative modifications in the arterial wall.[3,4] Oxidized LDLs exhibit *in vitro* a variety of biological properties potentially involved in atherogenesis, such as lipid accumulation in macrophagic cells (foam cell formation), overexpression of adhesion proteins (recruitment of mononuclear cells), local immune response alteration (inflammatory response), smooth muscle cell migration and proliferation, production of extracellular matrix, alterations of coagulation pathways, disturbance in the arterial tone regulation, cytotoxicity (apoptosis or necrosis).[4,5] These biological responses triggered by oxidized LDLs are mediated by various intracellular signaling pathways, and (dys-)regulation of gene

*Corresponding author. Tel.: +33-561-32-20-59. Fax: +33-561-32-29-84. E-mail: anesalv@toulouse.inserm.fr; salvayre@toulouse.inserm.fr

ISSN 1071-5762 print/ISSN 1029-2470 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10715760410001665244

expression.[5–7] This led Hajjar and Haberland[6] to consider oxidized LDLs as "trojan horses" because bioactive lipids internalized inside the cell trigger (dys-)regulation of various intracellular signaling pathways and of gene expression.[5,6]

The inflammatory response is thought to play a major role in the atherogenesis and in the trigger of plaque erosion or rupture. NF-κB is a central player in the regulation of both inflammatory response and cell survival. It is activated by a variety of factors (e.g. cytokines, oxidants) and also by oxidized LDLs. In fact, oxidized LDLs trigger a biphasic effect on NF-κB, depending on their concentration.[6] NF-κB is rapidly activated in vascular cultured cells by low concentrations of oxidized LDLs, under conditions inducing mitogenic and proinflammatory responses.[8] In contrast, longer periods of incubation and high oxidized LDLs concentrations impair NF-κB activation mediated by various agonists, and may thereby induce proapoptotic and immunosuppressive effects.[9] So far, how oxidized LDLs modulate cell signaling and mechanisms of NF-κB activation, is only partly understood.

This review is an attempt to give a synthetic view of the effects of oxidized LDLs on the NF-κB system, the mechanisms of activation and inhibition and the potential involvement in atherosclerosis.

THE NF-κB SYSTEM

The transcription factor NF-κB is an ubiquitous transcription factor that can be activated by a large number of extracellular stimuli such as cytokines, chemokines, growth factors, bacterial or viral products, hypoxia–reperfusion and stress generating responses.[10–12] NF-κB activation triggers the induction of inflammatory genes and may thereby play a role in the initiation and progression of various chronic inflammatory diseases and atherosclerosis.[9,10]

The NF-κB Family

The Rel/NF-κB family consists of the members NF-κB-1 (p50/p105), NF-κB-2 (p52/p100), p65 (RelA), RelB and c-Rel which form various homodimers and heterodimers, where the most common active form is p50 or p52/p65.[10–12] A characteristic feature of NF-κB proteins is that all the family members share a highly conserved Rel homology domain (RHD). This domain, composed of approximately 300 amino acid residues, contains a nuclear localization sequence (NLS) and is responsible for dimerization and DNA binding. In addition, the RHD is the site for the binding of the NF-κB inhibitors, IκBs.

Mechanisms of NF-κB Activation

The IκB family is constituted of (at least) seven members that share multiple copies of the ankyrin motif. IκBα, IκBβ, IκBε, IκBγ/p100, IκBδ/p105 and Bcl-3 control the activity of NF-κB. In resting cells, the ankyrin repeats of IκBs mask the NLS of NF-κB and prevent its nuclear translocation. Upon activation, IκB is rapidly phosphorylated on two serine residues (ser-32 and ser-36 in IκBα, ser-19 and ser-23 in IκBβ). This leads to IκB ubiquitination and subsequent degradation by the 26S proteasome. The released NF-κB dimer then translocates to the nucleus where it binds to specific promoters and activates the transcription of a wide variety of target genes (Fig. 1), including genes of cytokines (IL-1, TNF-α), adhesion molecules (VCAM-1, ICAM-1, E-selectin), regulators of apoptosis (c-IAPs, Bcl-xL), immunoreceptors (Table I).[13,14]

The critical regulatory step in the activation of NF-κB is the phosphorylation of the N-terminal regulatory serines of IκB proteins which is mediated by a high molecular weight IκB kinase complex (IKK) (approximately 700 kDa).[15–17] IKK is a serine-specific kinase composed of at least three subunits: two catalytic subunits IKKα (IKK-1) and IKKβ (IKK-2) and a regulatory subunit IKKγ. The predominant form of IKK is an heterodimer IKKα/IKKβ associated with either dimer or trimer of IKKγ. A fourth protein, IKAP (IKK complex-associated protein) may also be involved in IKK activation, but its physiological function is not clearly understood.

The inactive (non phosphorylated) IKK complex is activated by phosphorylation mediated by an heterogeneous group of serine/threonine kinases, named "IKK-kinases". This group includes members of the mitogen-activated protein kinase kinase kinase (MAPKKK) family such as NIK (NF-κB inducing kinase) and MEKK-1, -2 and -3 (MAPK/ERK kinase kinase),[17] protein kinase C (PKC),[18] AKT/protein kinaseB[19] and two related IκB-kinase IKKi/IKKε and NAK/TBK1 (NF-κB activating kinase/Tank binding kinase 1)[20] (Fig. 1).

In response to upstream stimuli, IKK-kinases are activated and recruited to the complex via IKKγ. Activated IKK-kinases induce both phosphorylation of the catalytic subunits and activation of the IKK complex, which in turn phosphorylates IκB. IKK activation is transient and is regulated by a C-terminal autophosphorylation and inactivation by phosphatases.[15,16] A second mechanism of autoregulation of the NF-κB/IκB system is also induced by NF-κB itself which elicits the synthesis of its own inhibitor IκB. The newly synthetized IκBα proteins enter the nucleus to bind activated NF-κB and carry it to the cytoplasm, thereby terminating the activation of gene expression.

It may be noted that NF-κB can be activated independently of the IKK complex by two alternative mechanisms. The first one is UV radiation-induced NF-κB activation to which does not involve IκBα phosphorylation. The second exception is anoxia, which stimulates phosphorylation of IκBα

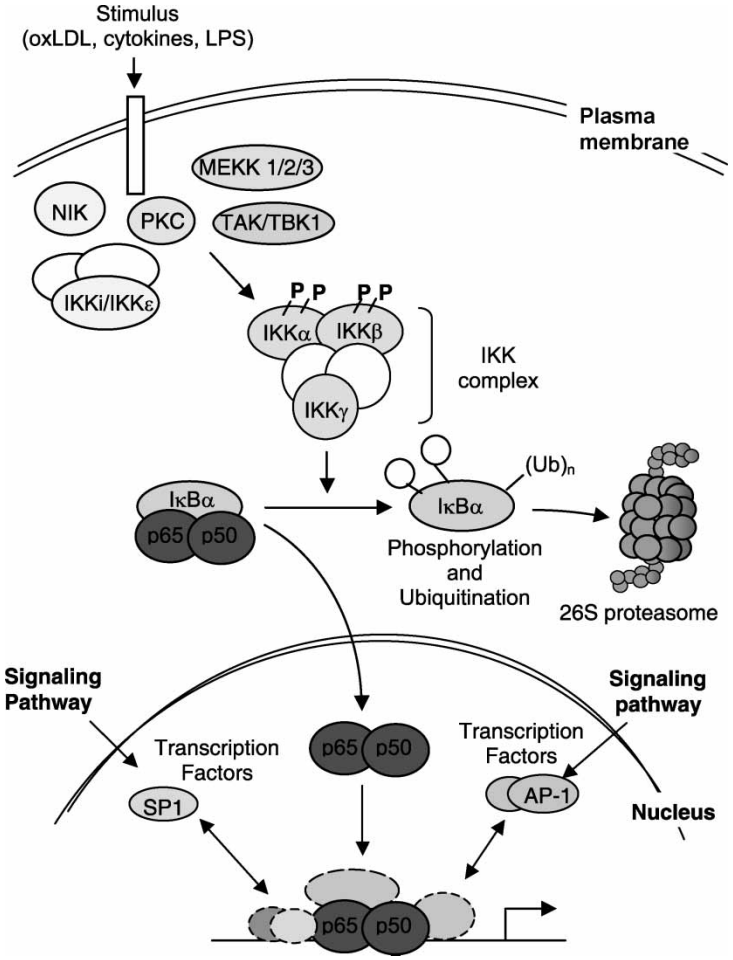

FIGURE 1 NF-κB signaling pathway.

at tyrosine 42, that may bind the SH2 domain of PI3-kinase which pulls it away from NF-κB.[15–17]

NF-κB Activation by Oxidative Stress

NF-κB is a redox-sensitive transcription factor which is activated by intracellular reactive oxygen species (ROS), such as H_2O_2, superoxide anion, hydroxyl radicals. This hypothesis is supported by the activating effect of exogenous H_2O_2 in various cell types and by the inhibitory effect of antioxidants, including both chemical antioxidants (such as NAC or PDTC) and overexpressed antioxidant enzymes.[21–24]

TABLE I NF-κB target genes

Proinflammatory cytokines	IL-1 α–β, IL-2, IL-6, IL-12, TNF-α, LTα, LTβ, IFNβ, GM-CSF, M-CSF (Macrophage colony stimulating Factor), G-CSF (Granulocyte colony stimulating Factor)
Chemokines	IL-8, MiP-1 α (Macrophage inflammatory protein 1 α), MCP-1 (Macrophage chemotactic protein 1), RANTES, Eotaxine, Gro α, β, γ
Adhesion molecules	ICAM-I, VCAM-1, E-selectin
Acute phase proteins	SAA (Serum Amyloid A)
Inflammatory enzymes	iNOS (inducible Nitric Oxyde Synthase), COX-2 (cyclooxygenase-2), 5-lipoxygenase, cytosolic phospholipase A_2
Receptors	Interleukin-2 receptor (α-chain), T-cell receptor (β-chain), Platelet-activating factor receptor (PAF-R)
Regulators of apoptosis	c-IAP-1, c-IAP-2, A1 (BFl-1), Bcl-X_L, Fas ligand, Fas (CD95), c-myc, p53, cycline D1, A20
Protein of coagulation	Tissue factor

See reviews: (Barnes, *New England J. of Medicine*, 1997—Ghost and Karin, *Cell*, 2002—May and Ghost, *Immunology Today*, 1998).

The role played by ROS in the mechanism of NF-κB activation remains only poorly understood.[21] ROS could act as second messengers that modulate the kinase activities of NF-κB signaling pathways, IKK complex activation[22] and/or DNA binding and transactivation activity of the NF-κB dimers. This role remains still cell and stimulus specific.[23,24]

NF-κB, OXIDIZED LDLs AND ATHEROSCLEROSIS

Several lines of evidence suggest that NF-κB could contribute to the initiation and progression of atherosclerosis. Activated NF-κB (which is not detected in the normal arterial wall), is present in areas predisposed to atherosclerotic lesion formation, in atherosclerotic lesions and in plaques of patients with unstable angina pectoris.[25–28] The presence of activated NF-κB in atherosclerotic areas raises the question of the potential role of NF-κB in atherogenesis, since a local inflammatory process is implicated in atherogenesis and NF-κB is known to be implicated in the inflammatory response.

Hypercholesterolemia and Oxidized LDLs are Associated with NF-κB Activation

Several reports indicate that NF-κB activation is associated with hyperlipidemia or oxidized LDLs. For instance, mice placed on an atherogenic diet or injected with oxidized LDLs exhibited an hepatic accumulation of lipid peroxides, associated with NF-κB activation in the liver and the expression of a set of inflammatory and oxidative stress responsive genes, such as serum amyloid A (SAA), heme oxygenase and JE (the murine homologue of Macrophage Chemotactic protein-1, MCP-1).[29,30] In coronary arteries of hypercholesterolemic pigs, activated NF-κB colocalizes with areas of intense lipid staining.[31] In a rat model, injected LDLs induced NF-κB activation and expression of intracellular cell adhesion molecule-1 (ICAM-1) in aortic endothelium.[32] It may be noted that a causal link between NF-κB activation and early atherosclerotic lesions has not been firmly demonstrated so far, because genetically engineered mice deficient in p65 or IκBα die during embryonic development.[33]

Oxidized LDLs Components Involved in the Modulation of NF-κB Activity

Various bioactive oxidized lipids present in oxidized LDLs are able to trigger NF-κB activation.

13-HPODE (13-hydroperoxyoctadecadienoic acid) induces NF-κB activation and subsequent overexpression of the vascular cell adhesion molecule-1 (VCAM-1) in porcine vascular SMCs.[34] This effect is mediated by oxidative stress and PKC as assessed by the inhibition of NF-κB DNA binding and transcription of VCAM-1 gene by the antioxidant NAC and calphostin C.[34]

Oxidized phospholipids isolated from minimally oxidized LDL induced monocyte binding on endothelium and increase in VCAM-1 expression.[35,36]

Lysophosphatidylcholine (Lyso-PC) elicited PKC and tyrosine kinases activation and subsequent NF-κB activation and overexpression of ICAM-1, VCAM-1 and MCP-1.[37–40]

Oxysterols (7-keto, 7-hydroxy and 25-hydroxycholesterol are the major oxysterols present in oxidized LDLs) are thought to be responsible for a large part of the biological effects of oxidized LDLs on cultured cells, for instance, regulation of cholesterol metabolism, apoptosis, inflammation, immunosuppression and atherogenesis.[41] To date NF-κB activation by oxysterol has not been reported, but cannot be excluded *a priori*, since oxysterols induce a cellular oxidative stress (which is known to trigger NF-κB activation) and trigger nuclear receptors LXRα and LXRβ activation (which are often activated concomitantly with NF-κB).[42–44]

Hydroxynonenal (HNE) and other aldehydic products, formed during oxidation of polyunsaturated fatty acids (PUFA) of LDLs, are able to trigger or modulate cell signaling involved in proliferation or apoptosis.[45] Low HNE concentration induces MAPK activation and AP-1 nuclear binding, but does not activate NF-κB in these conditions.[46–49] Moreover, HNE has been shown to downregulate NF-κB nuclear binding and counteract lipopolysaccharide (LPS)-induced NF-κB activation.[49]

Mechanism of NF-κB Activation Induced by Oxidized LDLs

Moderate concentrations of oxidized LDLs trigger a rapid and dose-dependent activation of NF-κB.[50] Oxidized LDLs trigger the activation of the PI-3K/Akt pathway which is involved in cell survival.[51] The PI-3K/Akt pathway has been shown to associate with and activate the IKK complex[52] and to increase the transactivation potential of NF-κB (p65).[53] However, in smooth muscle cells overexpressing mutated forms of PI-3K and AKT, oxLDLs are still able to induce NF-κB activation, thus suggesting that other signaling pathways may be involved in the oxLDL-mediated NF-κB nuclear translocation.[46] This activation is concomitant with cell signaling leading to cell proliferation or death and may constitute a defense mechanism against apoptosis.

Another survival mechanism that regulates cell death and proliferation induced by oxidized LDLs

is the generation of bioactive sphingolipid metabolites, for instance sphingosine-1-phosphate (S1-P).[54] S1-P is involved in NF-κB activation mediated by inflammatory cytokines.[55,56] The mechanism of S1-P-induced NF-κB activation is poorly defined, but could involve calcium mobilization and oxidative stress.[57] However, it may be noted that the oxidized LDL-induced S1-P generation in smooth muscle cells[58] is apparently dispensable for NF-κB activation, since S1-P inhibitors did not abrogate the oxidized LDL-induced NF-κB activation.[46]

ROS are also implicated in the oxidized LDL-induced NF-κB activation, as assessed by the inhibitory effect of antioxidants.[46,59,60] ROS are generated by a DPI-sensitive oxidase system activated through signaling triggered by binding of oxidized LDLs to LOX-1 (lectin-like ox-LDL receptor-1) in endothelial cells, since an anti-LOX-1 antibody inhibits significantly NF-κB activation.[59,60] How oxidized LDL-induced ROS activate NF-κB is not completely elucidated, and various mechanisms may be involved. A first mechanism may implicate the PI-3K/Akt pathway which is activated by oxidized LDLs[51] and is able to activate the IKK complex and NF-κB (p65).[52,53] Another mechanism may involve MAPK and the serine-threonine kinase RSK (pp90rsk). The serine-threonine kinase RSK (pp90rsk), a downstream target of MAPK, plays a role in the regulation of a number of cellular functions and cell survival via association and phosphorylation of signaling proteins, such as Bad and IκBα, and of transcriptional regulators including c-Fos, estrogen receptor, NF-κB, cAMP-response element-binding protein (CREB) and CREB-binding protein.[61,62] As oxidized LDLs are able to activate MAPK,[63] it may be hypothesized that they may also activate RSK (a well known downstream target of MAPK). However, RSK activation by oxidized LDLs has not been reported so far.

Finally, the ubiquitin/proteasome system is also required for IκB degradation and NF-κB nuclear translocation triggered by oxidized LDLs.[46]

NF-κB Inhibition by Oxidized LDLs

Unexpectedly, oxidized LDLs induce a biphasic effect on NF-κB, since short term incubation of cells with oxidized LDLs activated NF-κB, whereas long term incubation prevented NF-κB activation triggered by pro-inflammatory agents.[9] For instance, oxidized LDLs inhibit the LPS-induced NF-κB activation and subsequent expression of TNF-α, IL-1β and PAF-receptor in macrophages.[9,64–67] In the same way, oxidized LDLs suppress the phytohaemagglutinin (PHA)-induced NF-κB activation and subsequent IL-2 receptor expression in human T-lymphocytes.[68]

Several bioactive lipids formed during LDL oxidation and present in oxidized LDLs could participate in this inhibitory effect, either by down-regulating signaling pathways implicated in the pro-inflammatory response or by activating antagonistic pathways.

Lyso-phosphatidylcholine (LPC) also regulates NF-κB activity in a biphasic manner dependent on incubation time and concentration (NF-κB being activated by low concentrations, but inhibited by high concentrations),[37] similar to that observed with oxidized LDLs.[9] The inhibitory effect of LPC was associated with a decreased expression of pro-inflammatory and pro-thrombotic proteins.[69]

Oxidized phospholipids have been shown to reduce the endotoxin-induced tissue damages and to down-regulate LPS-induced cyclooxygenase-2 expression in human macrophages, possibly by targeting both ERK and NF-κB pathways.[67,70]

4-Hydroxy-2-nonenal (HNE), aldehydic molecules generated during peroxidation of PUFA, is also able to inhibit NF-κB activation. For instance, HNE prevents the LPS-induced IκBα phosphorylation in THP-1 monocytic cells,[49] probably by reacting with and inhibiting IKK.[71] HNE (like oxidized LDLs) is also able to inhibit the proteasome activity and subsequent proteolysis of IκBα, possibly through a modification of proteasome proteins by 4-HNE.[72,73] Finally, both mechanisms may converge to inhibit NF-κB activation and subsequent expression of adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin, in human aortic endothelial cells.[74]

Oxidized PUFAs, such as 9-HODE and 13-HODE, prostaglandin J2 and HETE, have been identified as endogenous ligands and activators of PPARγ that are able to reduce NF-κB activation.[75–77] PPARα and PPARγ are expressed in atherosclerotic lesions and can reduce adhesion molecules expression and recruitment of leukocytes to endothelial cells, regulate lipid metabolism in macrophagic cells, inhibit the proliferation and migration of smooth muscle cells and modulate the inflammatory response.[77,78] PPARs antagonize NF-κB activation by several converging mechanisms, including negative regulation of NF-κB, overexpression of IκBα and of antioxidant enzymes such as catalase.[76,79,80]

Oxysterols have been shown to inhibit NF-κB activation elicited by pro-inflammatory agents.[65] This inhibitory effect could be mediated by transcription factors of the LXR and Sp families. LXRs are oxysterol-activated nuclear receptors that regulate genes controlling lipid homeostasis and inflammation.[81,82] The anti-inflammatory effect of activated LXR is mediated, at least in part, through interference with the NF-κB signaling pathway.[83] Oxysterols are also able to regulate the activity of members of the Sp-family of transcription factors[84] that are important regulators

of inflammatory processes.[85] This family, belonging to the zinc-finger family of DNA binding proteins, consists of four proteins designed Sp1 (specific protein 1), Sp2, Sp3 and Sp4 which are general transcription factors that recognise GC and/or GT box motifs in the promoter of many ubiquitous or tissue specific genes.[85] *In vitro* studies of the TNF-α promoter suggested that Sp1 and Sp3 may either compete with NF-κB for binding to the promoter or interact with DNA-bound NF-κB.[84]

Potential Consequences of Oxidized LDLs-induced NF-κB Modulation

NF-κB plays a central role in host defense and inflammatory response, as well as in apoptosis regulation.[86] Oxidized LDLs are able to alter the regulation, thereby altering the pro-/anti-inflammatory and pro-/anti-apoptotic balance in atherosclerotic plaques.[87]

NF-κB in Apoptosis and Survival

The analysis of RelA−/−, IKKβ−/− and IKKγ −/− mice and cell biology studies have clearly shown that NF-κB is an anti-apoptotic transcription factor.[86] This also has been clearly demonstrated in the case of oxidized LDLs, using transfection of synthetic double-stranded DNA that binds and inhibits the transcriptional factor NF-κB and thereby potentiates the pro-apoptotic effect induced by oxidized LDLs.[88] The anti-apoptotic activity of NF-κB is dependent on induction of genes[86] such as members of the Bcl-2 family (A1, Bcl-xL), cellular inhibitors of apoptosis (c-IAPs), FLICE inhibitory protein (cFLIP), TNRF-associated factors TRAF1 and TRAF2 and A20 protein.[86] These proteins act as inhibitors of both the death-receptor pathway (TRAF, cFLIP) and the mitochondrial apoptotic pathway (Bcl-2 related proteins, IAPs).[86] Of note, most of the apoptosis inducers, such as oxidized LDLs, cytokines, TNF-α and chemotherapeutic agents, activate NF-κB which in turn modulates the pro-apoptotic signaling.[86,89,90] Reversely, during apoptosis, activated caspases cleave several anti-apoptotic proteins including NF-κB itself, the NF-κB regulatory proteins IKK, IκBα (converted into a super-repressor-like molecule) and NF-κB-regulated anti-apoptotic factors. This caspase-mediated proteolysis tends to eliminate the protection conveyed by NF-κB survival pathway.[86] In conclusion, NF-κB is therefore at the crossroads of life and death and plays a critical role in it by regulating the level of anti-apoptotic proteins.[86]

Although little evidence exists for a pro-apoptotic function of NF-κB,[86] in some specific cases, NF-κB could induce the overexpression of various pro-apoptotic genes. For instance, in endothelial cells, NF-κB may enhance the pro-apoptotic effect of oxidized LDLs by up-regulating LOX-1 expression (LOX-1 promoter containing a consensus NF-κB binding site),[91,92] thereby increasing the uptake of oxidized LDLs and the subsequent toxicity. Another example is the oxidized LDL-induced NF-κB-mediated overexpression of Angiotensin Receptor-1 (AT1) which may up-regulate LOX-1 expression and enhances the toxicity of oxidized LDLs[93] and alter the balance between cell survival/apoptosis.[94,95] Finally, NF-κB may also up-regulate the expression of pro-apoptotic genes such as Fas ligand, Fas (Cd95), c-myc and p53, thereby sensitizing cells to apoptotic stimuli.[96–98]

Adhesion Molecules and Cytokines

During atherogenesis, dyslipidemia and lipid deposition in the intima are coupled to a local inflammatory response, involving endothelial cells, monocytes and lymphocytes.[99,100] Oxidized LDLs and other pro-inflammatory stimuli trigger a NF-κB-mediated overexpression of adhesion molecules (VCAM-1, ICAM-1, E-selectin) that are involved in the "rolling" and adhesion of leukocytes to endothelial cells.[101] Moreover, oxidized LDL also induced the expression of other pro-inflammatory genes of chemoattractant chemokines (MCP-1), growth factors (M-CSF, GM-CSF) and inflammatory cytokines (IL-1, TNF-α).[102] As shown in Table I, these proteins of inflammation are, in part, under the control of NF-κB.

Nitric Oxide (NO) Synthase and Endothelial Dysfunction

NO exhibits anti-inflammatory properties which is associated with inhibition of the expression of adhesion molecule (VCAM-1, ICAM-1, E-selectin) and cytokines (IL-8 and IL-6).[100,103,104] This is mediated (at least in part) through inhibition (by NO) of NF-κB activation, resulting either from overexpression and stabilization of the NF-κB inhibitor IκBα[105] and/or from a decreased DNA binding activity of NF-κB dimers subsequent to the modification of the redox-sensitive Cys62 residue of p50 subunit.[106] It may be noted that NO production (and subsequent anti-inflammatory and anti-atherogenic effects) are inhibited by oxidized LDLs and oxidized lipids.[107–109]

Tissue Factor

Tissue factor (TF) is a potent pro-thrombotic protein that acts as a cofactor for activated factor VII (FVIIa) and induces thereby thrombin generation.[110,111] It is expressed in vascular cells (monocytes, VSMCs, endothelial cells) in response to inflammatory cytokines, mitogens, endotoxins and initiates

rapidly the coagulation process in case of vascular injury.[111,112] TF gene expression is regulated by a number of transcription factors that bind two distinct enhancers in the TF promoter. A distal enhancer, containing two AP-1 sites and one κB site controls TF expression mediated by LPS or cytokines, whereas a proximal enhancer, containing three overlapping Egr-1/Sp1 binding sites, controls TF expression induced by serum or phorbol ester. The concerted action of these different transcription factors allow to express TF in a cell- and stimulus-specific manner.[112] It may be noted that oxidized LDLs and lipids can induce a biphasic effect on NF-κB activation and, subsequently, on TF level. This dual effect could explain apparently conflicting data reported in the literature. For instance, Brand *et al*.[113] demonstrated that oxidized LDLs significantly enhanced TF expression induced by LPS in human monocytes, whereas other reports have shown that lysoPC induce a decrease in both NF-κB activation and TF expression induced by LPS.[69]

Increased expression of TF is observed in all stages of atherosclerotic lesions, and plaques expressing the highest levels of TF are associated with the highest thrombotic risk.[114] As oxidized lipids up-regulate TF expression in vascular cells[115–118] and inactivate the anti-coagulant function of TFIP (tissue factor inhibitor protein),[119,120] this results in an imbalance between TF and TFIP activities and, subsequently, in an increased thrombogenicity.

CONCLUSION

The biological responses induced by oxidized LDLs and mediated through NF-κB-regulated genes, are sometimes apparently puzzling, because of the dual effects of oxidized LDLs, summarized in Table II. This dual effect of oxidized LDLs may essentially result from two mechanisms: (i) modulation of the balance between activation and inactivation of NF-κB (dependent on concentration of oxidized lipids and time of contact with the cells, inflammatory status of cells); (ii) stimulation by activated NF-κB of both the anti- and pro-apoptotic genes.

Activated NF-κB mediates, at least in part, the proinflammatory effects of oxidized LDLs, leading to the overexpression of adhesion molecules and chemokines, and subsequent recruitment and retention of mononuclear cells in the intima. This effect may be of particular importance in the early steps of atherogenesis and in the progression of atherosclerotic lesions.[2–5,27,99,100] NF-κB activation also participates in the regulation of cell survival, and in the balance between proliferation and apoptosis induced by oxidized LDLs. On one hand, activation of NF-κB by oxidized LDLs activates anti-apoptotic genes that counterbalance the toxicity of oxidized LDLs, but, on the other hand, oxidized LDLs also induce a NF-κB-mediated up-regulation of LOX-1 and AT-1 receptors expression, which may enhance the pro-apoptotic effect of oxidized LDLs and angiotensin, respectively. Interestingly, both chronic and acute inflammatory sites co-exist with localized apoptosis at sites of plaque rupture, thus suggesting that the anti-apoptotic effect of NF-κB is unable to counterbalance the pathogenic effect of local inflammatory conditions in which NF-κB is implicated.[27,78,100]

In contrast, oxidized LDLs are also able to reduce NF-κB activation induced by pro-inflammatory agents including LPS or cytokines. This inhibitory effect of oxidized LDLs may reduce the recruitment of leukocytes in the vascular wall, minimize the inflammatory response of activated T-lymphocytes and monocytes, impair the immune response against infections and limit TF expression. Down-regulation of the local immune responses can finally limit the inflammatory burst and maintain low level of inflammation which is a characteristic of the atherosclerotic lesions. But, on the other hand,

TABLE II Potential pathophysiological role of NF-κB in atherosclerotic lesions

(A) Potential consequences of NF-κB activation by oxLDL	
Targets of NF-κB	Potential physiological consequences
Expression of adhesion molecules, chemotactic proteins, inflammatory cytokines	Inflammation
Up-regulation of Lox-1 and AT-II receptors	Apoptosis of endothelial cells Endothelial dysfunction
Survival pathway	Cell survival Plaque stability
(B) *Potential consequences of NF-κB inhibition by oxLDL*	
Targets of NF-κB	Potential patho-physiological consequences
Reduced expression of inflammatory molecules (adhesion molecules, cytokines)	Immunosuppressive effect
Inhibition of Tissue Factor	Reduction of the pro-thrombotic state
Inhibition of iNOS expression and NO production	Pro- or anti-atherogenic effect depending on the local inflammatory status

NF-κB inhibition reduces the expression of anti-apoptotic proteins, thereby sensitizing cells to toxic stimuli and increasing the instability of the plaque.

During the progression of atherosclerotic plaque, NF-κB activation and the subsequent expression of anti-apoptotic proteins may favor healing and plaque stability, but may also lead to excessive fibro-proliferative process and luminal narrowing. Reversely, the inhibition of NF-κB and subsequent anti-apoptotic proteins may elicit plaque instability and increase the risk of plaque erosion/rupture and subsequent athero-thrombosis.

The definition of the overall conditions that actually favour either the pro-atherogenic or the anti-atherogenic side of NF-κB needs futher investigation.

Acknowledgements

We would like to thank Prof. G. Poli for valuable suggestions and careful reading of the manuscript. This work was supported by grants from INSERM, MESR University Toulouse-3 and GLN (Groupe lipide et Nutrition) to U-466. F. Robbesyn was a recipient of a fellowship from Fondation pour la Recherche Médicale.

References

[1] Stary, H.C. (1990) "The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life", *Eur. Heart J.* **11**, 3–19.
[2] Lusis, A.J. (2000) "Atherosclerosis", *Nature* **407**, 233–241.
[3] Ross, R. (1993) "The pathogenesis of atherosclerosis: a perspective for the 1990s", *Nature* **362**, 801–809.
[4] Witztum, J.L. and Steinberg, D. (1991) "Role of oxidized low density lipoprotein in atherogenesis", *J. Clin. Investig.* **88**, 1785–1792.
[5] Napoli, C., de Nigris, F. and Palinski, W. (2001) "Multiple role of reactive oxygen species in the arterial wall", *J. Cell. Biochem.* **82**, 674–682.
[6] Hajjar, D.P. and Haberland, M.E. (1997) "Lipoprotein trafficking in vascular cells. Molecular Trojan horses and cellular saboteurs", *J. Biol. Chem.* **272**, 22975–22978.
[7] Salvayre, R., Auge, N., Benoist, H. and Negre-Salvayre, A. (2002) "Oxidized low-density lipoprotein-induced apoptosis", *Biochim. Biophys. Acta* **1585**, 213–221.
[8] Berliner, J.A., Navab, M., Fogelman, A.M., Frank, J.S., Demer, L.L., Edwards, P.A., Watson, A.D. and Lusis, A.J. (1995) "Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics", *Circulation* **91**, 2488–2496.
[9] Brand, K., Eisele, T., Kreusel, U., Page, M., Page, S., Haas, M., Gerling, A., Kaltschmidt, C., Neumann, F.J., Mackman, N., Baeurele, P.A., Walli, A.K. and Neumeier, D. (1997) "Dysregulation of monocytic nuclear factor-kappa B by oxidized low-density lipoprotein", *Arterioscler. Thromb. Vasc. Biol.* **17**, 1901–1909.
[10] Ghosh, S. and Karin, M. (2002) "Missing pieces in the NF-kappaB puzzle", *Cell* **109**, S81–S96.
[11] May, M.J. and Ghosh, S. (1998) "Signal transduction through NF-kappa B", *Immunol. Today* **19**, 80–88.
[12] Barnes, P.J. and Karin, M. (1997) "Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases", *N. Engl. J. Med.* **336**, 1066–1071.
[13] Valen, G., Yan, Z.Q. and Hansson, G.K. (2001) "Nuclear factor kappa-B and the heart", *J. Am. Coll. Cardiol.* **38**, 307–314.
[14] Ross, J.S., Stagliano, N.E., Donovan, M.J., Breitbart, R.E. and Ginsburg, G.S. (2001) "Atherosclerosis and cancer: common molecular pathways of disease development and progression", *Ann. N.Y. Acad. Sci.* **947**, 271–292, discussion 292, 273.
[15] Karin, M. (1999) "The beginning of the end: IkappaB kinase (IKK) and NF-kappaB activation", *J. Biol. Chem.* **274**, 27339–27342.
[16] Zandi, E. and Karin, M. (1999) "Bridging the gap: composition, regulation, and physiological function of the IkappaB kinase complex", *Mol. Cell. Biol.* **19**, 4547–4551.
[17] Karin, M. and Delhase, M. (2000) "The I kappa B kinase (IKK) and NF-kappa B: key elements of proinflammatory signalling", *Semin. Immunol.* **12**, 85–98.
[18] Sanz, L., Sanchez, P., Lallena, M.J., Diaz-Meco, M.T. and Moscat, J. (1999) "The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation", *EMBO J.* **18**, 3044–3053.
[19] Ozes, O.N., Mayo, L.D., Gustin, J.A., Pfeffer, S.R., Pfeffer, L.M. and Donner, D.B. (1999) "NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase", *Nature* **401**, 82–85.
[20] Peters, R.T. and Maniatis, T. (2001) "A new family of IKK-related kinases may function as I kappa B kinase kinases", *Biochim. Biophys. Acta* **1471**, M57–M62.
[21] Bowie, A. and O'Neill, L.A. (2000) "Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries", *Biochem. Pharmacol.* **59**, 13–23.
[22] Kamata, H., Manabe, T., Oka, S., Kamata, K. and Hirata, H. (2002) "Hydrogen peroxide activates IkappaB kinases through phosphorylation of serine residues in the activation loops", *FEBS Lett.* **519**, 231–237.
[23] Schoonbroodt, S. and Piette, J. (2000) "Oxidative stress interference with the nuclear factor-kappa B activation pathways", *Biochem. Pharmacol.* **60**, 1075–1083.
[24] Janssen-Heininger, Y.M., Poynter, M.E. and Baeuerle, P.A. (2000) "Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB", *Free Radic. Biol. Med.* **28**, 1317–1327.
[25] Brand, K., Page, S., Rogler, G., Bartsch, A., Brandl, R., Knuechel, R., Page, M., Kaltschmidt, C., Baeuerle, P.A. and Neumeier, D. (1996) "Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion", *J. Clin. Investig.* **97**, 1715–1722.
[26] Hajra, L., Evans, A.I., Chen, M., Hyduk, S.J., Collins, T. and Cybulsky, M.I. (2000) "The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation", *Proc. Natl Acad. Sci. USA* **97**, 9052–9057.
[27] Ritchie, M.E. (1998) "Nuclear factor-kappaB is selectively and markedly activated in humans with unstable angina pectoris", *Circulation* **98**, 1707–1713.
[28] Wilson, S.H., Best, P.J., Edwards, W.D., Holmes, D.R., Jr., Carlson, P.J., Celermajer, D.S. and Lerman, A. (2002) "Nuclear factor-kappaB immunoreactivity is present in human coronary plaque and enhanced in patients with unstable angina pectoris", *Atherosclerosis* **160**, 147–153.
[29] Liao, F., Berliner, J.A., Mehrabian, M., Navab, M., Demer, L.L., Lusis, A.J. and Fogelman, A.M. (1991) "Minimally modified low density lipoprotein is biologically active *in vivo* in mice", *J. Clin. Investig.* **87**, 2253–2257.
[30] Liao, F., Andalibi, A., deBeer, F.C., Fogelman, A.M. and Lusis, A.J. (1993) "Genetic control of inflammatory gene induction and NF-kappa B-like transcription factor activation in response to an atherogenic diet in mice", *J. Clin. Investig.* **91**, 2572–2579.
[31] Wilson, S.H., Caplice, N.M., Simari, R.D., Holmes, D.R., Jr., Carlson, P.J. and Lerman, A. (2000) "Activated nuclear factor-kappaB is present in the coronary vasculature in experimental hypercholesterolemia", *Atherosclerosis* **148**, 23–30.
[32] Calara, F., Dimayuga, P., Niemann, A., Thyberg, J., Diczfalusy, U., Witztum, J.L., Palinski, W., Shah, P.K., Cercek, B., Nilsson, J. and Regnstrom, J. (1998) "An animal model to study local oxidation of LDL and its biological

effects in the arterial wall", *Arterioscler. Thromb. Vasc. Biol.* **18**, 884–893.
[33] Beg, A.A., Sha, W.C., Bronson, R.T., Ghosh, S. and Baltimore, D. (1995) "Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B", *Nature* **376**, 167–170.
[34] Natarajan, R., Reddy, M.A., Malik, K.U., Fatima, S. and Khan, B.V. (2001) "Signaling mechanisms of nuclear factor-kappab-mediated activation of inflammatory genes by 13-hydroperoxyoctadecadienoic acid in cultured vascular smooth muscle cells", *Arterioscler. Thromb. Vasc. Biol.* **21**, 1408–1413.
[35] Subbanagounder, G., Leitinger, N., Schwenke, D.C., Wong, J.W., Lee, H., Rizza, C., Watson, A.D., Faull, K.F., Fogelman, A.M. and Berliner, J.A. (2000) "Determinants of bioactivity of oxidized phospholipids. Specific oxidized fatty acyl groups at the sn-2 position", *Arterioscler. Thromb. Vasc. Biol.* **20**, 2248–2254.
[36] Subbanagounder, G., Watson, A.D. and Berliner, J.A. (2000) "Bioactive products of phospholipid oxidation: isolation, identification, measurement and activities", *Free Radic. Biol. Med.* **28**, 1751–1761.
[37] Sugiyama, S., Kugiyama, K., Ogata, N., Doi, H., Ota, Y., Ohgushi, M., Matsumura, T., Oka, H. and Yasue, H. (1998) "Biphasic regulation of transcription factor nuclear factor-kappaB activity in human endothelial cells by lysophosphatidylcholine through protein kinase C-mediated pathway", *Arterioscler. Thromb. Vasc. Biol.* **18**, 568–576.
[38] Kume, N., Cybulsky, M.I. and Gimbrone, M.A., Jr. (1992) "Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells", *J. Clin. Investig.* **90**, 1138–1144.
[39] Takahara, N., Kashiwagi, A., Maegawa, H. and Shigeta, Y. (1996) "Lysophosphatidylcholine stimulates the expression and production of MCP-1 by human vascular endothelial cells", *Metabolism* **45**, 559–564.
[40] Zhu, Y., Lin, J.H., Liao, H.L., Verna, L. and Stemerman, M.B. (1997) "Activation of ICAM-1 promoter by lysophosphatidylcholine: possible involvement of protein tyrosine kinases", *Biochim. Biophys. Acta* **1345**, 93–98.
[41] Brown, A.J. and Jessup, W. (1999) "Oxysterols and atherosclerosis", *Atherosclerosis* **142**, 1–28.
[42] Lizard, G., Gueldry, S., Sordet, O., Monier, S., Athias, A., Miguet, C., Bessede, G., Lemaire, S., Solary, E. and Gambert, P. (1998) "Glutathione is implied in the control of 7-ketocholesterol-induced apoptosis, which is associated with radical oxygen species production", *FASEB J.* **12**, 1651–1663.
[43] Alberti, S., Steffensen, K.R. and Gustafsson, J.A. (2000) "Structural characterisation of the mouse nuclear oxysterol receptor genes LXRalpha and LXRbeta", *Gene* **243**, 93–103.
[44] Leonarduzzi, G., Sottero, B. and Poli, G. (2002) "Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects (review)", *J. Nutr. Biochem.* **13**, 700–710.
[45] Leonarduzzi, G., Arkan, M.C., Basaga, H., Chiarpotto, E., Sevanian, A. and Poli, G. (2000) "Lipid oxidation products in cell signaling", *Free Radic. Biol. Med.* **28**, 1370–1378.
[46] Robbesyn, F., Garcia, V., Auge, N., Vieira, O., Frisach, M.F., Salvayre, R. and Negre-Salvayre, A. (2003) "HDL counterbalance the proinflammatory effect of oxidized LDL by inhibiting intracellular reactive oxygen species rise, proteasome activation, and subsequent NF-kappaB activation in smooth muscle cells", *FASEB J.* **17**, 543–745.
[47] Parola, M., Robino, G., Marra, F., Pinzani, M., Bellomo, G., Leonarduzzi, G., Chiarugi, P., Camandola, S., Poli, G., Waeg, G., Gentilini, P. and Dianzani, M.U. (1998) "HNE interacts directly with JNK isoforms in human hepatic stellate cells", *J. Clin. Investig.* **102**, 1942–1950.
[48] Camandola, S., Scavazza, A., Leonarduzzi, G., Biasi, F., Chiarpotto, E., Azzi, A. and Poli, G. (1997) "Biogenic 4-hydroxy-2-nonenal activates transcription factor AP-1 but not NF-kappa B in cells of the macrophage lineage", *Biofactors* **6**, 173–179.
[49] Page, S., Fischer, C., Baumgartner, B., Haas, M., Kreusel, U., Loidl, G., Hayn, M., Ziegler-Heitbrock, H.W., Neumeier, D. and Brand, K. (1999) "4-Hydroxynonenal prevents NF-kappaB activation and tumor necrosis factor expression by inhibiting IkappaB phosphorylation and subsequent proteolysis", *J. Biol. Chem.* **274**, 11611–11618.
[50] Maziere, C., Auclair, M., Djavaheri-Mergny, M., Packer, L. and Maziere, J.C. (1996) "Oxidized low density lipoprotein induces activation of the transcription factor NF kappa B in fibroblasts, endothelial and smooth muscle cells", *Biochem. Mol. Biol. Int.* **39**, 1201–1207.
[51] Auge, N., Garcia, V., Maupas-Schwalm, F., Levade, T., Salvayre, R. and Negre-Salvayre, A. (2002) "Oxidized LDL-induced smooth muscle cell proliferation involves the EGF receptor/PI-3 kinase/Akt and the sphingolipid signaling pathways", *Arterioscler. Thromb. Vasc. Biol.* **22**, 1990–1995.
[52] Romashkova, J.A. and Makarov, S.S. (1999) "NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling", *Nature* **401**, 86–90.
[53] Madrid, L.V., Mayo, M.W., Reuther, J.Y. and Baldwin, A.S., Jr. (2001) "Akt stimulates the transactivation potential of the RelA/p65 Subunit of NF-kappa B through utilization of the Ikappa B kinase and activation of the mitogen-activated protein kinase p38", *J. Biol. Chem.* **276**, 18934–18940.
[54] Cuvillier, O., Pirianov, G., Kleuser, B., Vanek, P.G., Coso, O.A., Gutkind, S. and Spiegel, S. (1996) "Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate", *Nature* **381**, 800–803.
[55] Xia, P., Wang, L., Gamble, J.R. and Vadas, M.A. (1999) "Activation of sphingosine kinase by tumor necrosis factor-alpha inhibits apoptosis in human endothelial cells", *J. Biol. Chem.* **274**, 34499–34505.
[56] Xia, P., Gamble, J.R., Rye, K.A., Wang, L., Hii, C.S., Cockerill, P., Khew-Goodall, Y., Bert, A.G., Barter, P.J. and Vadas, M.A. (1998) "Tumor necrosis factor-alpha induces adhesion molecule expression through the sphingosine kinase pathway", *Proc. Natl Acad. Sci. USA* **95**, 14196–14201.
[57] Shatrov, V.A., Lehmann, V. and Chouaib, S. (1997) "Sphingosine-1-phosphate mobilizes intracellular calcium and activates transcription factor NF-kappa B in U937 cells", *Biochem. Biophys. Res. Commun.* **234**, 121–124.
[58] Auge, N., Nikolova-Karakashian, M., Carpentier, S., Parthasarathy, S., Negre-Salvayre, A., Salvayre, R., Merrill, A.H., Jr. and Levade, T. (1999) "Role of sphingosine 1-phosphate in the mitogenesis induced by oxidized low density lipoprotein in smooth muscle cells via activation of sphingomyelinase, ceramidase, and sphingosine kinase", *J. Biol. Chem.* **274**, 21533–21538.
[59] Cominacini, L., Pasini, A.F., Garbin, U., Davoli, A., Tosetti, M.L., Campagnola, M., Rigoni, A., Pastorino, A.M., Lo Cascio, V. and Sawamura, T. (2000) "Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappaB through an increased production of intracellular reactive oxygen species", *J. Biol. Chem.* **275**, 12633–12638.
[60] Cominacini, L., Rigoni, A., Pasini, A.F., Garbin, U., Davoli, A., Campagnola, M., Pastorino, A.M., Lo Cascio, V. and Sawamura, T. (2001) "The binding of oxidized low density lipoprotein (ox-LDL) to ox-LDL receptor-1 reduces the intracellular concentration of nitric oxide in endothelial cells through an increased production of superoxide", *J. Biol. Chem.* **276**, 13750–13755.
[61] Shimamura, A., Ballif, B.A., Richards, S.A. and Blenis, J. (2000) "Rsk1 mediates a MEK-MAP kinase cell survival signal", *Curr. Biol.* **10**, 127–135.
[62] Frodin, M. and Gammeltoft, S. (1999) "Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction", *Mol. Cell. Endocrinol.* **151**, 65–77.
[63] Auge, N., Pieraggi, M.T., Thiers, J.C., Negre-Salvayre, A. and Salvayre, R. (1995) "Proliferative and cytotoxic effects of mildly oxidized low-density lipoproteins on vascular smooth-muscle cells", *Biochem. J.* **309**, 1015–1020.
[64] Ares, M.P., Kallin, B., Eriksson, P. and Nilsson, J. (1995) "Oxidized LDL induces transcription factor activator protein-1 but inhibits activation of nuclear factor-kappa B in human vascular smooth muscle cells", *Arterioscler Thromb. Vasc. Biol.* **15**(10), 1584–1590.

[65] Ohlsson, B.G., Englund, M.C., Karlsson, A.L., Knutsen, E., Erixon, C., Skribeck, H., Liu, Y., Bondjers, G. and Wiklund, O. (1996) "Oxidized low density lipoprotein inhibits lipopolysaccharide-induced binding of nuclear factor-kappaB to DNA and the subsequent expression of tumor necrosis factor-alpha and interleukin-1beta in macrophages", *J. Clin. Investig.* **98**, 78–89.
[66] Hourton, D., Stengel, D., Chapman, M.J. and Ninio, E. (2001) "Oxidized low density lipoproteins downregulate LPS-induced platelet-activating factor receptor expression in human monocyte-derived macrophages: implications for LPS-induced nuclear factor-kappaB binding activity", *Eur. J. Biochem.* **268**, 4489–4496.
[67] Bochkov, V.N. and Leitinger, N. (2003) "Anti-inflammatory properties of lipid oxidation products", *J. Mol. Med.* **81**, 613–626.
[68] Caspar-Bauguil, S., Tkaczuk, J., Haure, M.J., Durand, M., Alcouffe, J., Thomsen, M., Salvayre, R. and Benoist, H. (1999) "Mildly oxidized low-density lipoproteins decrease early production of interleukin 2 and nuclear factor kappaB binding to DNA in activated T-lymphocytes", *Biochem. J.* **337**, 269–274.
[69] Engelmann, B., Zieseniss, S., Brand, K., Page, S., Lentschat, A., Ulmer, A.J. and Gerlach, E. (1999) "Tissue factor expression of human monocytes is suppressed by lysophosphatidylcholine", *Arterioscler. Thromb. Vasc. Biol.* **19**, 47–53.
[70] Eligini, S., Brambilla, M., Banfi, C., Camera, M., Sironi, L., Barbieri, S.S., Auwerx, J., Tremoli, E. and Colli, S. (2002) "Oxidized phospholipids inhibit cyclooxygenase-2 in human macrophages via nuclear factor-kappaB/IkappaB- and ERK2-dependent mechanisms", *Cardiovasc. Res.* **55**, 406415.
[71] Ji, C., Kozak, K.R. and Marnett, L.J. (2001) "IkappaB kinase, a molecular target for inhibition by 4-hydroxy-2-nonenal", *J. Biol. Chem.* **276**, 18223–18228.
[72] Vieira, O., Escargueil-Blanc, I., Jurgens, G., Borner, C., Almeida, L., Salvayre, R. and Negre-Salvayre, A. (2000) "Oxidized LDLs alter the activity of the ubiquitin-proteasome pathway: potential role in oxidized LDL-induced apoptosis", *FASEB J.* **14**, 532–542.
[73] Okada, K., Wangpoengtrakul, C., Osawa, T., Toyokuni, S., Tanaka, K. and Uchida, K. (1999) "4-Hydroxy-2-nonenal-mediated impairment of intracellular proteolysis during oxidative stress. Identification of proteasomes as target molecules", *J. Biol. Chem.* **274**, 23787–23793.
[74] Uchida, K. (2003) "4-Hydroxy-2-nonenal: a product and mediator of oxidative stress", *Prog. Lipid Res.* **42**, 318–343.
[75] Nagy, L., Tontonoz, P., Alvarez, J.G., Chen, H. and Evans, R.M. (1998) "Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma", *Cell* **93**, 229–240.
[76] Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J. and Glass, C.K. (1998) "The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation", *Nature* **391**, 79–82.
[77] Duval, C., Chinetti, G., Trottein, F., Fruchart, J. and Staels, B. (2002) "The role of PPARs in atherosclerosis", *Trends Mol. Med.* **8**, 422–430.
[78] Mallat, Z. and Tedgui, A. (2000) "Apoptosis in the vasculature: mechanisms and functional importance", *Br. J. Pharmacol.* **130**, 947–962.
[79] Delerive, P., Gervois, P., Fruchart, J.C. and Staels, B. (2000) "Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-alpha activators", *J. Biol. Chem.* **275**, 36703–36707.
[80] Klucis, E., Crane, D. and Masters, C. (1984) "Sequential alterations in the micro-localization of catalase in mouse liver after treatment with hypolipidemic drugs", *Mol. Cell. Biochem.* **65**, 73–82.
[81] Edwards, P.A., Kennedy, M.A. and Mak, P.A. (2002) "LXRs; oxysterol-activated nuclear receptors that regulate genes controlling lipid homeostasis", *Vascul. Pharmacol.* **38**, 249–256.
[82] Joseph, S.B., Castrillo, A., Laffitte, B.A., Mangelsdorf, D.J. and Tontonoz, P. (2003) "Reciprocal regulation of inflammation and lipid metabolism by liver X receptors", *Nat. Med.* **9**, 213–219.
[83] Castrillo, A., Joseph, S.B., Marathe, C., Mangelsdorf, D.J. and Tontonoz, P. (2003) "Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages", *J. Biol. Chem.* **278**, 10443–10449.
[84] Englund, M.C., Karlsson, A.L., Wiklund, O., Bondjers, G. and Ohlsson, B.G. (2001) "25-hydroxycholesterol induces lipopolysaccharide-tolerance and decreases a lipopolysaccharide-induced TNF-alpha secretion in macrophages", *Atherosclerosis* **158**, 61–71.
[85] Suske, G. (1999) "The Sp-family of transcription factors", *Gene* **238**, 291–300.
[86] Karin, M. and Lin, A. (2002) "NF-kappaB at the crossroads of life and death", *Nat. Immunol.* **3**, 221–227.
[87] De Martin, R., Hoeth, M., Hofer-Warbinek, R. and Schmid, J.A. (2000) "The transcription factor NF-kappa B and the regulation of vascular cell function", *Arterioscler. Thromb. Vasc. Biol.* **20**, 83–88.
[88] Nishio, E., Kanda, Y., Kuroki, Y. and Watanabe, Y. (2000) "The potentiation of oxLDL induced apoptosis by inhibition of NF-kappaB", *Life Sci.* **67**, 1983–1992.
[89] Zen, K., Karsan, A., Stempien-Otero, A., Yee, E., Tupper, J., Li, X., Eunson, T., Kay, M.A., Wilson, C.B., Winn, R.K. and Harlan, J.M. (1999) "NF-kappaB activation is required for human endothelial survival during exposure to tumor necrosis factor-alpha but not to interleukin-1beta or lipopolysaccharide", *J. Biol. Chem.* **274**, 28808–28815.
[90] Liu, H., Lo, C.R. and Czaja, M.J. (2002) "NF-kappaB inhibition sensitizes hepatocytes to TNF-induced apoptosis through a sustained activation of JNK and c-Jun", *Hepatology* **35**, 772–778.
[91] Li, D. and Mehta, J.L. (2000) "Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells: evidence from use of antisense LOX-1 mRNA and chemical inhibitors", *Arterioscler. Thromb. Vasc. Biol.* **20**, 1116–1122.
[92] Aoyama, T., Sawamura, T., Furutani, Y., Matsuoka, R., Yoshida, M.C., Fujiwara, H. and Masaki, T. (1999) "Structure and chromosomal assignment of the human lectin-like oxidized low-density-lipoprotein receptor-1 (LOX-1) gene", *Biochem. J.* **339**, 177–184.
[93] Li, D., Saldeen, T., Romeo, F. and Mehta, J.L. (2000) "Oxidized LDL upregulates angiotensin II type 1 receptor expression in cultured human coronary artery endothelial cells: the potential role of transcription factor NF-kappaB", *Circulation* **102**, 1970–1976.
[94] Nickenig, G. (2002) "Central role of the AT(1)-receptor in atherosclerosis", *J. Hum. Hypertens* **16**(Suppl. 3), S26–S33.
[95] Bascands, J.L., Girolami, J.P., Troly, M., Escargueil-Blanc, I., Nazzal, D., Salvayre, R. and Blaes, N. (2001) "Angiotensin II induces phenotype-dependent apoptosis in vascular smooth muscle cells", *Hypertension* **38**, 1294–1299.
[96] Hsu, S.C., Gavrilin, M.A., Lee, H.H., Wu, C.C., Han, S.H. and Lai, M.Z. (1999) "NF-kappa B-dependent Fas ligand expression", *Eur. J. Immunol.* **29**, 2948–2956.
[97] Chan, H., Bartos, D.P. and Owen-Schaub, L.B. (1999) "Activation-dependent transcriptional regulation of the human Fas promoter requires NF-kappaB p50–p65 recruitment", *Mol. Cell. Biol.* **19**, 2098–2108.
[98] Qin, Z.H., Chen, R.W., Wang, Y., Nakai, M., Chuang, D.M. and Chase, T.N. (1999) "Nuclear factor kappaB nuclear translocation upregulates c-Myc and p53 expression during NMDA receptor-mediated apoptosis in rat striatum", *J. Neurosci.* **19**, 4023–4033.
[99] Ross, R. (1999) "Atherosclerosis—an inflammatory disease", *N. Engl. J. Med.* **340**, 115–126.
[100] Libby, P. (2002) "Inflammation in atherosclerosis", *Nature* **420**, 868–874.
[101] Berliner, J.A., Territo, M.C., Sevanian, A., Ramin, S., Kim, J.A., Bamshad, B., Esterson, M. and Fogelman, A.M. (1990) "Minimally modified low density lipoprotein stimulates monocyte endothelial interactions", *J. Clin. Investig.* **85**, 1260–1266.
[102] Rajavashisth, T.B., Andalibi, A., Territo, M.C., Berliner, J.A., Navab, M., Fogelman, A.M. and Lusis, A.J. (1990)

"Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins", *Nature* **344**, 254–257.
[103] De Caterina, R., Libby, P., Peng, H.B., Thannickal, V.J., Rajavashisth, T.B., Gimbrone, Jr., M.A., Shin, W.S. and Liao, J.K. (1995) "Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines", *J. Clin. Investig.* **96**, 60–68.
[104] Peng, H.B., Spiecker, M. and Liao, J.K. (1998) "Inducible nitric oxide: an autoregulatory feedback inhibitor of vascular inflammation", *J. Immunol.* **161**, 1970–1976.
[105] Peng, H.B., Libby, P. and Liao, J.K. (1995) "Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B", *J. Biol. Chem.* **270**, 14214–14219.
[106] Matthews, J.R., Botting, C.H., Panico, M., Morris, H.R. and Hay, R.T. (1996) "Inhibition of NF-kappaB DNA binding by nitric oxide", *Nucleic Acids Res.* **24**, 2236–2242.
[107] Yang, X., Cai, B., Sciacca, R.R. and Cannon, P.J. (1994) "Inhibition of inducible nitric oxide synthase in macrophages by oxidized low-density lipoproteins", *Circ. Res.* **74**, 318–328.
[108] Hattori, Y., Hattori, S. and Kasai, K. (2001) "4-Hydroxynonenal prevents NO production in vascular smooth muscle cells by inhibiting nuclear factor-kappaB-dependent transcriptional activation of inducible NO synthase", *Arterioscler. Thromb. Vasc. Biol.* **21**, 1179–1183.
[109] Huang, A., Li, C., Kao, R.L. and Stone, W.L. (1999) "Lipid hydroperoxides inhibit nitric oxide production in RAW264.7 macrophages", *Free Radic. Biol. Med.* **26**, 526–537.
[110] Carmeliet, P. and Collen, D. (1998) "Tissue factor", *Int. J. Biochem. Cell. Biol.* **30**, 661–667.
[111] Tremoli, E., Camera, M., Toschi, V. and Colli, S. (1999) "Tissue factor in atherosclerosis", *Atherosclerosis* **144**, 273–283.
[112] Mackman, N. (1995) "Regulation of the tissue factor gene", *FASEB J.* **9**, 883–889.
[113] Brand, K., Banka, C.L., Mackman, N., Terkeltaub, R.A., Fan, S.T. and Curtiss, L.K. (1994) "Oxidized LDL enhances lipopolysaccharide-induced tissue factor expression in human adherent monocytes", *Arterioscler. Thromb.* **14**, 790–797.
[114] Ardissino, D., Merlini, P.A., Arlens, R., Coppola, R., Bramucci, E., Lucreziotti, S., Repetto, A., Fetiveau, R. and Mannucci, P.M. (2000) "Tissue factor in human coronary atherosclerotic plaques", *Clin. Chim. Acta* **291**, 235–240.
[115] Lewis, J.C., Bennett-Cain, A.L., DeMars, C.S., Doellgast, G.J., Grant, K.W., Jones, N.L. and Gupta, M. (1995) "Procoagulant activity after exposure of monocyte-derived macrophages to minimally oxidized low density lipoprotein. Co-localization of tissue factor antigen and nascent fibrin fibers at the cell surface", *Am. J. Pathol.* **147**, 1029–1040.
[116] Drake, T.A., Hannani, K., Fei, H.H., Lavi, S. and Berliner, J.A. (1991) "Minimally oxidized low-density lipoprotein induces tissue factor expression in cultured human endothelial cells", *Am. J. Pathol.* **138**, 601–607.
[117] Cui, M.Z., Penn, M.S. and Chisolm, G.M. (1999) "Native and oxidized low density lipoprotein induction of tissue factor gene expression in smooth muscle cells is mediated by both Egr-1 and Sp1", *J. Biol. Chem.* **274**, 32795–32802.
[118] Bochkov, V.N., Mechtcheriakova, D., Lucerna, M., Huber, J., Malli, R., Graier, W.F., Hofer, E., Binder, B.R. and Leitinger, N. (2002) "Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca(++)/NFAT", *Blood* **99**, 199–206.
[119] Horie, S., Hiraishi, S., Hirata, Y., Kazama, M. and Matsuda, J. (2000) "Oxidized low-density lipoprotein impairs the anticoagulant function of tissue-factor-pathway inhibitor through oxidative modification by its high association and accelerated degradation in cultured human endothelial cells", *Biochem. J.* **352**, 277–285.
[120] Horie, S., Hiraishi, S., Hamuro, T., Kamikubo, Y. and Matsuda, J. (2002) "Oxidized low-density lipoprotein associates strongly with carboxy-terminal domain of tissue factor pathway inhibitor and reduces the catalytic activity of the protein", *Thromb. Haemost.* **87**, 80–85.